mGlu stoichiometry and to explore how these receptors cooperatively activate in response to ligand binding. We first used single molecule fluorescence photobleaching in live Xenopus oocytes and in receptors immobilized from mammalian cell lysate to map the dimer interfaces of homo and heterodimers. This work was complemented by experiments that use a covalently attached photoswitchable tethered ligand (PTL) that works as an agonist to analyze the cooperativity that arises from ligand binding to one or both subunits within a dimer. Finally, intersubunit FRET was used to analyze the conformational changes of mGluRs in order to gain an integrative view of receptor assembly, structural dynamics, and activation.

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Glycan-Based Connectivity Regulates the Hierarchical Organization of Membrane Receptors by Coupling their Micro- and Nano-Scale Lateral Mobility

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Glycan-protein interactions are emerging as important modulators of membrane protein organization and dynamics, regulating multiple cellular functions. In particular, it has been postulated that glycan-mediated interactions regulate surface residence time of glycoproteins and endocytosis. How this precisely occurs is poorly understood. We applied a combination of super-resolution nanoscopy and single molecule-based approaches to study the role of glycan-based interactions on the dynamics of the glycosylated pathogen recognition receptor DC-SIGN, at the nano- and micrometer scale. We find that cell surface glycan-mediated interactions do not influence the nanoscale lateral organization of DC-SIGN in nanoclusters but restrict the mobility of the receptor to distinct micron-size membrane regions. These meso-scale regions are in turn enriched by the endocytic protein clathrin, thereby dynamically promoting DC-SIGN transient nano-scale arrest and interaction with clathrin. Disruption of glycan-based connectivity leads to larger membrane exploration, reduced clathrin interaction and compromised clathrin-dependent internalization of virus-like particles. Therefore, our work uncovers a novel mechanism through which glycan-protein interactions act as decision-makers in fine-tuning membrane-related functions by dynamically coupling micro- and nanoscale receptor lateral mobility, thus adding a new layer of regulation to the hierarchical spatiotemporal organization of the cell membrane.

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The Role of Ligand Density in the Binding of Von Willebrand Factor by the Glycoprotein Ib-IX-V Complex in Platelets

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The initial arrest of platelets at a wound site requires the binding of glycoprotein Ib-IX-V complex (GPIb-IX-V) to the extracellular matrix protein von Willebrand factor (VWF). Increasing forces on these bonds increases the bond lifetime, known as a catch bond. Recently, we have shown that platelets are able to transmit cytoskeletal forces through the GPIbα subunit of GPIb-IX-V, which binds to the A1 domain of VWF. This provides an internal force on the bond to maintain adhesion in the absence of external forces. Integrin engagement and force transmission are known to require receptor clustering. We therefore investigate whether the clustering of GPIb-IX-V receptor is needed for the transmission of cytoskeletal forces to VWF. We first examined whether GPIb-IX-V clusters formed in spreading platelets. GPIbα-positive punctate structures were visualized by confocal microscopy and correlated with dark regions imaged using interference reflection microscopy (IRM), indicating that GPIb-IX-V forms adhesive contacts that are similar in size to integrin-related focal adhesions in platelets. To investigate the effect of ligand density on platelet spreading, we prepared molecularly-defined adhesive ligand spots, which were separated 28 or 108 nm apart by nonadhesive regions using self-assembling gold nanoparticles. These nanoparticles were coated with A1 domain of VWF. When seeded on nanoparticles with 28 nm spacing, platelets appeared well-spread and formed punctate adhesions. However, when seeded on 108 nm spacing, platelet adhesion was significantly reduced and those platelets that did adhere spread poorly. These findings demonstrate that an upper limit for ligand density exists where platelet adhesion and spreading are impeded. Investigating the effects of ligand spacing will facilitate an understanding of GPIbα clustering in platelet adhesion and spreading, thus providing insight into thrombotic diseases and congenital bleeding disorders.

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Chelidonine Interferes with IL-6R/STAT3 Signaling in Uveal Melanoma Cells

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There is increasing evidence suggesting the importance of IL-6 in oncogenesis: it stimulates tumor cells proliferation and promotes cell survival through the inhibition of apoptosis. IL-6 acts on a receptor complex consisting of the cytokine-specific IL-6Rα chain and the signal-transducing gp130 subunit. Binding of IL-6 to IL-6Rα induces dimerization of gp130 which initiates multiple signaling cascades, including STAT3 activation.
Chelidonine, the major alkaloid component of C. majus, provokes cell death in a variety of tumor cells, possibly through the antiapoptotic Bcl-2 protein. Expression of Bcl-2 is upregulated by STAT3 activation, which is thought to be responsible for IL-6-mediated survival of tumor cells.
Herein we aimed to study the effect of chelidonine on the viability of human uveal melanoma cells as wells as its interference with the IL-6R/STAT3 signaling pathway.
Antiproliferative and cell death-inducing effects of chelidonine were assessed by flow cytometry. The apoptotic potential of chelidonine was followed by DNA fragmentation and PI exclusion/annexin V binding assays. Expression of STAT3, Bcl-2 and IL-6Rα and the efficiency of STAT3 activation was also studied by flow cytometry.
Combined analysis of cell death experiments revealed chelidonine-induced apoptosis of UM cells. Moreover, alkaloid treatment also resulted in necrotic cell death.
Pretreatment of cells even with sublethal doses of chelidonine led to the appearance of a subpopulation with abrogated STAT3 activation upon IL-6 stimulation and modified Bcl-2 expression levels. We detected cells with reduced expression of STAT3 and IL-6Rα; however, the amount of these cells was significantly lower than that of cells with abolished STAT3 signaling.
According to our results chelidonine exerts its effect via a STAT3-dependent mechanism. Our findings imply the possible use of chelidonine in cancer therapy: it can either provoke cell death or weaken the antiapoptotic machinery of tumor cells fueled by IL-6.

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The Site of Arachidonic Acid Release Drives Calcium Dynamics in β-Cells

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Insulin is the blood glucose-lowering hormone essential for glucose homeostasis. β-Cells secrete insulin in pulses in response to glucose. Loss of the oscillatory nature of insulin secretion is associated with the development of type II diabetes. Calcium is the most important trigger of insulin release even though the exact mechanism of insulin secretion regulation by calcium is elusive. Arachidonic acid (AA) is an essential signalling molecule involved in regulation of physiological functions of many cell types. In insulin secreting pancreatic β-cells AA was shown to modulate calcium levels and as result to trigger insulin secretion. However, the exact interplay between AA signalling and insulin secretion is still a matter of speculation. In the present work we investigated the difference of AA action at the internal membranes (IMs) and the plasma membrane (PM) of β-cells. To perform this study, we developed a caging group which permits localization, visualization and quantitative photorelease of AA exclusively on the PM of living cells. We applied it in combination with a previously reported caging group used to release AA on the IMs. We found that the release of AA on the PM and the IMs leads to a significantly different modulation of intracellular calcium dynamics. Uncaging of AA on the PM induces calcium oscillations in non-oscillating cells and increases the duration of calcium transients in oscillating cells, leading to overall higher calcium levels. Release of AA on the IM results in transiently or permanently diminished calcium oscillations in β-cells and lower average calcium levels. We attribute the observed effects to direct action of AA on Arachidonic acid Regulated Calcium (ARC) channels localized on the PM and potentially a negative feedback mechanism triggered by higher levels of AA at the internal membranes.